Immunotoxins and central nervous system neoplasia

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The poor prognosis associated with central nervous system (CNS) malignancy has led investigators to seek new, innovative treatment modalities. Immunotoxins, carrier molecules linked to toxic agents, combine high specificity for tumor-associated antigens with extreme potency. The rationale for both the development of these compounds and for their application to CNS neoplasia is explained. This report discusses the design and construction of immunomolecules, using toxins that differ in their mechanism of action bound to ligands directed against various antigens. A comparison is made between the in vitro efficacy of standard chemotherapy and immunotoxins in glioblastoma- and medulloblastoma-derived cell lines. A review is included of the results of experiments in animals with leptomeningeal neoplasia, where prolongation of survival following intrathecal administration of immunotoxins has been reported. The obstacles encountered in clinical trials with other types of cancer are addressed and approaches to optimize the use of these novel agents in the context of treating malignant disease of the CNS are suggested.

Key Words • brain neoplasm • diphtheria toxin • immunotoxin • leptomeningeal tumor • monoclonal antibody • transferrin receptor

Advances in the conventional treatment of primary malignant tumors of the central nervous system (CNS), including surgery, radiation therapy, and systemic chemotherapy, have not improved the prognosis for this disease.92 The 2-year survival rate for glioblastoma multiforme is still less than 20%.33,139 Retrospective studies have demonstrated that the increase in survival rate observed when surgical resection was the only treatment modality may not persist if additional postoperative treatment is rendered, particularly external beam irradiation.92

With the advent of monoclonal antibody technology, reagents have become available that selectively recognize cell-surface antigens expressed on neoplastic rather than normal tissues.58,72,136,156,157 Over the last 10 years, a large number of monoclonal antibodies that show high specificity for various types of tumor cells have been prepared.51,53,58,85,93 Many of these antibodies have been chemically linked to drugs, radionuclides, and toxins in attempts to create more tumor selective therapeutic agents.4,12,16,26,69,76,77,80,155 The biological heterogeneity of malignant gliomas and the variable expression of antigens among and within tumors have complicated the development of tumor-specific antibody-directed therapy.7,17,146,156 Despite raising antibodies to human neuroectodermal tumor- and human glioma-associated antigens, polyclonal and monoclonal serological methodologies have not identified antigens specific for these tumor types.14,33 Nevertheless, the presence of semispecific antigens on the surface of malignant brain-tumor cells may prove useful for designing new therapeutic modalities.4,12,53,65,156,157

Immunotoxins, monoclonal antibodies, or other ligands directed against tumor-associated antigens covalently linked to toxic proteins constitute a new class of compounds designed to combine exquisite cell-type selectivity with extraordinary potency.12,25,40,58,76,77,107,116,156,157 The construction of immunotoxins is based on the "magic bullet" concept, linking toxic compounds to carrier molecules specific for a target cell, first introduced by Paul Ehrlich almost 100 years ago.27,58,107,116 Although only recently introduced to neurological surgery, immunotoxins have been in existence for 10 to 12 years.35,71,136,157 In vitro studies using immunotoxins have shown cell-type specificity and high potency for various neoplastic tissue-culture cells, including...
Definitions of Abbreviations

ADP = adenosine diphosphate
ADR = Adriamycin (doxorubicin)
CRM = cross-reacting material
DT = diphtheria toxin
EGF = epidermal growth factor
EGFR = epidermal growth factor receptor
HLA = human leukocyte antigen
Tr = transferrin
TrR = transferrin receptor

Rationale for Immunotoxin Therapy

Many substances kill cells by destroying membrane integrity, disrupting energy metabolism, or blocking deoxyribonucleic acid, ribonucleic acid (RNA), or protein synthesis. Most standard chemotherapeutic drugs depend on the higher rate of replication of neoplastic cells as compared to normal cells to obtain a cytotoxic effect, and have limited potency for tumors with low growth factors.58,130,134 The lack of specificity of systemic chemotherapy for malignant cells has led to dose-limiting side effects and unacceptable toxicity.58 With immunotoxins, as with traditional chemotherapy, the establishment of a therapeutic window in which the dose produces antitumor activity without producing toxic side effects is necessary for clinical utility. Guided by their cell-type specific component toward neoplastic cells, immunotoxins have a mechanism of action affecting protein synthesis that is different and potentially superior to that of most chemotherapeutic agents. Moreover, hypoxia, a factor responsible for the resistance of tumor cells to radiation therapy and chemotherapy, has no effect on the mechanism of action of immunotoxins.33,52 The type of natural or acquired resistance that many cancer cells develop to standard chemotherapy should not occur with toxin-based therapy.35,36,58

Most chemotherapeutic drugs act stoichiometrically to kill tumor cells, often requiring more than $10^4$ to $10^5$ molecules per cell to cause cell death. 23 Chlorambucil requires 20,000 alkylations to kill a single tumor cell and the alkylating agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) kills only 50% of human glioblastoma-derived tissue-culture cells at concentrations as high as $10^{-3}$ to $10^{-6}$ M 23,74,119 In contrast, intact ricin immunotoxins can achieve the same result at a concentration of $10^{-13}$ M.136 Exact comparisons are difficult because of differences in assay technique; however, the extreme potency of immunotoxins by seven to 10 orders of magnitude over BCNU is apparent. The higher efficiency of immunotoxins is explained by their catalytic activity, reacting repeatedly with many intracellular targets once the toxin has reached the cytosol. These toxic enzymes inactivate over 200 ribosomes or elongation factor-2's per minute, irrespective of the cell cycle or cellular division, with only a single molecule necessary for cell death (Table 1).78,128,140,148

Toxins Used to Produce Immunotoxins

The toxic moieties used to construct immunotoxins are natural by-products of bacteria, plants, or fungi, which in all cases enzymatically inactivate protein synthesis (Table 2).58,107,136 Plant toxins used in producing immunotoxins for CNS tumor treatment include ricin and abrin or their A chains, pokeweed antiviral protein, modeccin, gelonin, saporin, and momordica charantia inhibitor. Bacterial toxins include diphtheria toxin (DT), cross-reacting material (CRM) 107, and Pseudomonas aeruginosa exotoxin A. Alpha-sarcin is derived from a fungus. An extensive discussion of all toxins used in immunotoxin production is outside the scope of this article; emphasis will be placed only on those being developed for CNS use.

Most toxins, such as ricin, abrin, modeccin, and DT are composed of two polypeptide chains, an A chain and a B chain, linked by a disulfide bond. The 20- to 30-kD A chain, which causes cell death by inhibiting protein synthesis once inside the cytoplasm, is nontoxic to cells and animals.101 The B chain binds toxins nonspecifically to cell-surface receptors on most eukaryotic cells and promotes internalization or translocation of the A
chain into the cell.58,149,154 Pseudomonas aeruginosa exotoxin A has three separate domains on one polypeptide chain that are responsible for binding to the cell, translocation into the cell, and inhibition of protein synthesis, respectively. After binding to cell-surface receptors, peptide toxins are endocytosed into clathrin-coated pits and vesicles before they move into acidic endosomes (in the case of DT and P. aeruginosa exotoxin A) or neutral pH golgi (in the case of ricin) then translocate across the vesicle membrane into the cytosol.34,35,58,89,104,121,122,149 Many investigators have shown that immunotoxins constructed with the intact toxin have greater potency with more predictable cytotoxicity than A chain hybrids.9,43,129,135,137,153 The loss of potency seen with A chain immunotoxins may be attributed to the role of the B chain in facilitating toxin entry into the cytosol.

Ricin

Rcin, a 62-kD protein purified from castor bean seeds, has an A chain and a B chain linked by disulfide bonds.102,156 After entry into the cytosol, the A chain depurinates a single adenine base in the 28S ribosomal RNA of the 60S ribosomal subunit located near the site of elongation factor-2 binding, blocking protein synthesis and causing cell death.58 Chemotherapeutic drugs used to construct immunoconjugates have in- cluded the vinca alkaloids, methotrexate, and Adriamycin (ADR, doxorubicin).4,155 Adriamycin, a potent intercalating agent, has been covalently linked to a monoclonal antibody to create an effective in vitro and in vivo agent against a malignant glial neoplasm.155

Diphtheria Toxin

Diphtheria toxin is a potent protein with a molecular weight of 62 kD, secreted by Corynebacterium diphtheriae.107,147,148 The single chain that comprises DT must be proteolytically nicked at an arginine-rich site for the A and B subunits to be active against human cells.107,122 The A chain catalyzes the transfer of adenosine diphosphate (ADP)-ribose to elongation factor-2, preventing the translocation of peptidyl-t-RNA on ribosomes, thereby blocking protein synthesis and subsequently killing the cell.105 Entry of the A subunit into the cytosol is facilitated by the B chain. The DT receptor has not been characterized.33

Cross-Reacting Material 107

Cross-reacting material 107 is a new genetically engineered toxin that is identical to DT except for two amino acid substitutions in the B chain.49,71 These two-point mutations inactivate toxin binding 8000-fold, do not affect the translocation function, and increase the tumor-specific toxicity 10,000-fold for immunotoxins made with this toxin.49

Pseudomonas aeruginosa Exotoxin A

Pseudomonas aeruginosa exotoxin A has a molecular weight of 66 kD and is produced by the bacterium P. aeruginosa.35,107 The nature of the cell-surface receptor for P. aeruginosa exotoxin A is unknown. A low intracellular pH is required for translocation of the toxin into the cytoplasm. Three distinct structural domains have been identified by crystallographic analysis.3 Domain 3 at the C-terminus is responsible for the ADP-ribosylation of elongation factor-2 that results in cytotoxicity, an enzymatic activity similar to that of DT.3 Removal of binding domain 1 produces the residual protein PE 40 that has been used to make immunotoxins with little or no nonspecific binding.73

Other Toxins and Drugs

Pokeweed antiviral protein, abrin, saporin, modeccin, and gelonin function in a fashion similar to ricin by inactivating ribosomes and blocking protein synthesis.6,103 The fungal toxin alpha-sarcin inhibits protein synthesis by breaking a phosphodiester bond in the 28S ribosomal RNA of the 60S ribosomal subunit near the elongation factor-2 binding site.29,52 Chemotherapeutic drugs used to construct immunoconjugates have included the vinca alkaloids, methotrexate, and Adriamycin (ADR, doxorubicin).4,155 Adriamycin, a potent intercalating agent, has been covalently linked to a monoclonal antibody to create an effective in vitro and in vivo agent against a malignant glial neoplasm.155

Ligands Used in Producing Immunotoxins

In producing immunotoxins, the binding ligand is as functionally important as the toxic protein component of the molecule. Toxins have been cross-linked by a disulfide or more stable thioether bond156 to polyclonal antibodies, monoclonal antibodies, hormones, lectins, growth factors, and antigens to construct immunoconjugates. Mooylen and Cooperband49 created the first immunotoxin in 1970 when they linked DT to the immunoglobulin (Ig)A fraction of guinea pig antisera to mumps virus. The antibody-toxin conjugate was directed against monkey kidney cells that were infected with the mumps virus and that expressed viral surface
antigens. Although high in vitro potency was demonstrated, selectivity between antigen-positive and antigen-negative cells increased only twofold. Krolick, et al.,35 linked affinity-purified anti-idiotype polyclonal antibodies to the ricin A chain creating an immunotoxin against the mouse B-cell tumor BCL1, where all cells of the tumor expressed the same truly tumor-specific antigen. This antibody-ricin A chain conjugate killed 70% of tumor cells in vitro with little effect on non-antigen bearing cells. In 1977, Chang and Neville21 linked the human placental lactogen hormone by a disulfide bond to the DT A chain but were unable to demonstrate toxicity of the conjugate despite preservation of the A chain enzymatic activity. Other hormones used to construct immunotoxins have included insulin,94 human chorionic gonadotropin,97 thyrotropin-releasing hormone, and melanotropin (unpublished data). Epidermal growth factor (EGF) conjugated to the ricin A chain by Cawley, et al.,20 displayed toxicity to 3T3 cells, whereas EGF bound to the DT A chain had no cytotoxic effects. When using a growth factor as the toxin carrier, there is a potential problem that the ligand may act to promote cellular growth if the amount of growth factor-toxin conjugate administered is less than the lethal dose.35

Because of the high tissue specificity obtainable with monoclonal antibodies, most work has focused on their use as suitable binding ligands. The first immunotoxin constructed using a monoclonal antibody directed against a tumor-associated antigen was reported in 1980 by Gilliland, et al.43 Approximately 50% colorectal carcinoma cells were killed after 24 hours when exposed to conjugates composed of the ricin or DT A chain and a monoclonal antibody to a colorectal carcinoma tumor-associated antigen at concentrations of 10-9 M.43 High tissue specificity and high antigen affinity are features that make some monoclonal antibodies useful for immunotoxin development.111 Tumor-selective antibodies will bind 104 to 107 sites per cell in vitro and much less in vivo.150 Antibodies that are effectively internalized make better immunotoxins than those that are not, as demonstrated in ovarian cancer cells.111 Only a minority of monoclonal antibodies will yield an immunotoxin that is efficacious in animal models.10.58

Monoclonal antibody-directed toxin therapy should be targeted to cell-surface antigens or receptors that are produced in greater number on neoplastic cells than on normal cells. Although the function of most antigens is unknown, internalization of the antigen into an appropriate intracellular compartment with release of the toxin into the cytosol is necessary for cell killing. Unlike monoclonal antibody-linked high-energy radionuclides, which are cytotoxic when bound to the cell surface, toxins must enter the cell to cause an effect, and bystander cells are not influenced unless they express the same receptor or antigen on their surface.26.35

Potential cell membrane targets expressed on malignant tumors of the CNS in both cell culture and surgical samples are the EGF receptor (EGFR), a rearranged deletion-mutant tumor-specific EGFR, and the transferrin receptor (TrR).55.64.80 Libermann, et al.,78-80 demonstrated that glioblastomas multiforme amplify and overexpress the EGFR gene. Similarly, Humphrey, et al.,63 found EGFR gene amplification in biopsy specimens from six of eight human gliomas established as xenografts in nude mice. Using Scatchard analysis, affinity reactions, immunoprecipitation, Western immunoblots, and immunohistochemistry, they detected expression of the EGFR gene only in those xenografts demonstrating gene amplification.69 Amplification of the EGFR gene was seen by Helseth, et al.,77 in four of 16 glioblastomas, none of six astrocytomas, none of two oligodendrogliomas, one of three mixed gliomas, none of 11 meningiomas, and three of six brain metastases. Abnormal expression of the EGFR gene was detected by Gerosa, et al.,42 in four of five glioblastoma multiforme tissue-culture cell lines. Epidermal growth factor receptor binding was detected in 10 of 14 glioblastoma multiforme, one of nine low-grade astrocytomas, and five of six meningiomas by Whittle, et al.,144 In surgical specimens, Hall, et al.,25 found EGFR expression on all of four ependymomas and two of four glioblastomas multiforme using a competitive radioreceptor assay. The number of EGFR's per cell for the ependymomas ranged from 1000 to 6000. In reports by Whittle, et al., and Libermann, et al.,80 EGFR's were not present on the single primitive neuroectodermal tumor studied by each group.

The TrR, a mediator of cellular iron uptake, is expressed in greater number by dividing cells than by quiescent cells.132 The high requirement for iron by rapidly dividing cells, such as those of glioblastomas multiforme or medulloblastomas, suggest that immunotoxin therapy targeted to the TrR would not be affected by mechanisms of antigenic heterogeneity,17 antigenic modulation,59 or genetic loss.46 Transferrin receptors are not shed into the circulation and should not block anti-TrR-mediated cell killing.123 Efficient internalization of the TrR by cancer cells makes it an ideal target for immunotoxins.107.131

Zovickian, et al.,136 demonstrated increased expression of the TrR on glioblastoma- and medulloblastoma-derived cell lines and in surgical tissue samples of glioblastoma and medulloblastoma using a solid-phase indirect radioimmunoassay technique. Expression of the TrR on glioblastoma multiforme and medulloblastoma surgical tissue samples was estimated by Hall, et al.,35 by means of a radioreceptor assay. Using immunohistochemical technique, Recht, et al.,115 found staining intensity greater than 75% for the TrR on nine of 10 glioblastomas multiforme, one of two grade III anaplastic astrocytomas, and one low-grade glioma. Transferrin receptors were not detectable on normal brain tissue in any of these studies.35.115.120 Flow cytometric analysis was used by Recht, et al.,116 to demonstrate the presence of TrR on greater than 95% of cells from five human glioma cell lines using the anti-TrR monoclonal antibody 7D3. Also through the
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use of flow cytometry, Colombatti, et al., demonstrated human leukocyte antigen (HLA)-DR antigens and the glioma-associated antigen GE 2 on a glioblastoma cell line.

Monoclonal antibodies for cell-surface antigens on glioblastoma multiforme, which by themselves have cytotoxic reactivities against glioblastoma-derived tissue-culture cells, have been developed that may be useful for future immunotoxin construction. Monoclonal antibodies used to produce immunotoxins against glioma tissue-culture cells and glioma xenografts have been targeted to glioma-associated antigens, HLA-DR antigens, and Tr. The growth factor transferrin (Tr) has been conjugated to CRM 107 and the ricin A chain to yield potent immunotoxins against glioma cells, being 500 times more toxic than the A chain alone. The Tr-ricin A chain immunotoxin was over 100,000 times more potent than BCNU in reducing glioma cell survival in vitro.

An eightfold increase in toxicity to human glioma target cells of an immun conjugate of the mouse antihuman glioma monoclonal antibody SZ 39 and ADR, as compared with free ADR, was reported by Zhu, et al. The 50% inhibitory concentration of the SZ 39-ADR immun conjugate was 9.16 x 10^{-9} M for target cells compared to 7.08 x 10^{-8} M for ADR alone. The SZ 39-ADR conjugate was 11-fold less toxic than ADR alone against nontarget K562 human leukemia cells.

Among other cell types known to disseminate to the CNS, immunotoxins directed against both breast carcinoma and malignant melanoma may be clinically applicable. The 45A12-CRM 107 immunotoxin described by Johnson, et al., was particularly potent against breast carcinoma-derived tissue-culture cell lines. Godal, et al., found differential sensitivity of eight melanoma cell lines to immunotoxins composed of the monoclonal antimelanoma antibody 9.2.27 conjugated to abrin. The different inherent target cell sensitivities to abrin may reflect the cells' abilities to internalize and process the toxin.

In Vitro Application

The observed in vitro cytotoxic activity of immunotoxins against malignant CNS disease offers promise that these agents may eventually reach clinical utility. Zovickian, et al., reported that an antiTr-ricin immun conjugate, in the presence of the carboxylic ionophore monensin killed more than 50% of glioblastoma- and medulloblastoma-derived tissue-culture cells at a concentration of 5.6 x 10^{-10} M after 18 hours. The selective toxicity of the intact ricin-based immunotoxin against "target" cells compared to "nontarget" cells or normal brain tissue increased more than 150- to 1380-fold. Using an antiTr-ricin A chain immunotoxin, Recht, et al., demonstrated a 50% inhibition of protein synthesis at concentrations ranging from 1.9 x 10^{-9} to 1.8 x 10^{-8} M in five human glioma cell lines. Co-incubation with monensin increased the toxicity of the immunotoxin against the glioma cells 16- to 842-fold. The mechanism by which monensin accelerates the action of the ricin A chain is unclear.

Johnson, et al., reported a 10,000-fold increase in tumor-specific toxicity to glioblastoma- and medulloblastoma-derived cell lines using an immunotoxin made with CRM 107 as compared to free toxin. Cross-reacting material 107 coupled to human diferric Tr showed efficient killing of cell lines derived from medulloblastoma, glioblastoma multiforme, and breast carcinoma at concentrations between 3.9 x 10^{-10} and 1.1 x 10^{-10} M. Conjugates constructed using a monoclonal antibody to the human Tr 45A12 and CRM 107 or the ricin A chain demonstrated comparable potency but were not as effective as the Tr-CRM 107 conjugate. Cerebrospinal fluid from normal human and brain-tumor patients did not inhibit the in vitro activity of these reagents.

Colombatti, et al., completed in vitro studies of several antihuman glioma cytotoxic conjugates constructed from monoclonal antibodies to the GE 2 glioma-associated antigen, HLA-DR antigens, and Tr linked to ricin or the ricin A chain. Transferrin linked to the ricin A chain, co-incubated with monensin, demonstrated the highest cytotoxic activity for glioma cells, being 5000 times more toxic than the A chain alone. The Tr-ricin A chain immunotoxin was over 100,000 times more potent than BCNU in reducing glioma cell survival in vitro.

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in intracerebral human glioma xenografts treated with the intraperitoneal SZ 39-ADR immunoconjugate had a median survival time of 51 days compared to 36 days for those treated with ADR alone and 33 days for those in the negative control group that received phosphate-buffered saline.

Potential complications associated with the systemic delivery of immunotoxins may be avoided by direct delivery into a compartmentalized space such as the cerebrospinal fluid of the CNS or the intraperitoneal cavity. Intraperitoneal or intrathecal delivery can produce high local concentrations of the drug with a greater therapeutic effect. Several investigators have demonstrated in vivo efficacy of immunotoxins in the compartmentalized treatment of cancer. Watanabe, et al., showed prolonged survival, from 30 to 50 days, in a nude mouse model inoculated intraperitoneally with 10⁷ human pancreatic adenocarcinoma tissue-culture cells (T3M-4) and treated intraperitoneal with 60 µg of antitumor monoclonal antibody-ricin A chain immunotoxin at 2, 5, 7, and 10 days after inoculation. Gregg, et al., extended the median survival of strain-2 guinea pigs by 200% by administering 30 µg/kg of intraperitoneal anti-idiotype monoclonal antibody (M6)-intact ricin immunotoxin 24 hours after the intraperitoneal inoculation of 10⁵ L. C. B-cell leukemia cells. Using monoclonal antibodies to the murine TrR conjugated to recombinant ricin A chain, Bjorn and Groetsema prolonged survival in mice with ascitic P388 D1 lymphoid tumors following intraperitoneal treatment.

Zovickian and Youle extended survival in guinea pigs using M6-ricin immunotoxin delivered intrathecally 24 hours after delivery of L.C. leukemia cells into the cisterna magna. The results correspond to a 2 to 5 log kill of L.C. cells in the immunotoxin-treated animals. Intrathecal toxicity trials have shown that the maximum tolerated dose of Tr-CRM 107 immunotoxin in guinea pigs was 2 × 10^-9 M. This same dose of immunotoxin was nontoxic when administered intrathecally to rhesus monkeys, representing a concentration in the cerebrospinal fluid 20- to 5000-fold higher than the level that was found to be effective against neoplastic glial cells in vitro and suggesting that a significant therapeutic window may exist for immunotoxins used to treat leptomeningeal neoplasia.

**Clinical Trials**

In clinical trials, immunotoxins have been used primarily to eliminate T-cells from allogeneic bone-marrow grafts to prevent graft-versus-host disease. Systemic administration of immunotoxins against malignant disease has been studied in only a few Phase I and Phase II trials, and no reports of immunotoxin treatment of CNS tumors are available. Twenty-two patients with metastatic malignant melanoma were treated intravenously for up to 10 days by Spitler, et al., with a murine monoclonal anti-body-ricin A chain immunotoxin. The toxic side effects included hypoalbuminemia, peripheral edema, weight gain, malaise, anorexia, fever, and decreased electrocardiogram voltage. The half-life of the conjugate was 30 minutes, and most patients developed antibodies to the drug. One late complete response was seen.

In Phase I clinical trials directed against refractory breast carcinoma, nine women received intravenously an immunotoxin composed of recombinant ricin A chain linked to a monoclonal antibody that reacted with approximately 50% of breast carcinoma cell lines in vitro. Toxic side effects included capillary-leak syndrome and a severe sensorimotor peripheral neuropathy that developed in three patients 1 month after therapy, presumably due to cross-reactivity of the immunotoxin with Schwann cells. One partial response was seen, and antibody formation to the toxin and to the ligand was observed. Among 16 patients with metastatic colon cancer treated intravenously using an anti-72-kD glycoprotein-ricin A chain immunotoxin, Byers, et al., noted two partial responses. Antibody production to both the murine immunoglobulin and to the ricin A chain was observed.

At the present time, the only reports of leptomeningeal tumor treatment using immunotoxins involve labeled monoclonal antibodies. Moseley, et al., treated 15 patients with neoplastic meningitis with between 11 and 60 mCi of antibody. The tumors treated included one pineoblastoma, one B-cell lymphoma, one spinal teratoma, two melanomas, two glioblastomas multiforme, four medulloblastomas, and one lung, one ovarian, one breast, and one bladder carcinoma. In patients with glioblastoma and medulloblastoma, I-labeled antibodies were directed against a 230-kD gioma-associated glycoprotein and a primitive neuroectodermal-associated antigen, respectively. Seven of 15 patients experienced aseptic meningitis, manifesting as nausea, vomiting, and headache. Two patients had seizures and three of eight patients developed reversible bone-marrow suppression. Six patients sustained an objective response for 7 to 26 months. Three of four patients with medulloblastoma and both patients with gliomatosis had no response.

A Phase I clinical trial using anti-TrR-recombinant ricin A chain immunotoxin therapy for patients with leptomeningeal neoplasia due to lung carcinoma, breast carcinoma, and lymphoma is presently being conducted at the National Institutes of Health.

**Future Perspectives**

Because of the poor prognosis for malignant disease of the CNS and current therapy offering little prolongation of survival, new, innovative treatment modalities are needed. Immunotoxins, which have demonstrated in vitro efficacy against primary and metastatic neoplastic disease of the CNS, may represent such a therapeutic option. Traditional therapy may be best for cytoreduction, reserving immunotoxins for the treat-
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ment of residual tumor cells. Combination chemotherapy is generally more effective than single-agent therapy; antibody-toxin conjugates might successfully be combined with other chemotherapeutic agents or radiation therapy because of a mechanism of action different from that of standard cancer treatments. 3,8,39,133,141 The side effects of immunotoxin therapy, chemotherapy, and radiation therapy may therefore not be cumulative. 3,8,39 In a nude mouse model of colon cancer, it was recently shown that an immunotoxin administered with chemotherapy was more active than was either treatment alone. 108

Combinations of antibodies directed against the same antigen have inhibited tumor cell growth in vitro and in vivo in a synergistic manner. 142 In a similar fashion, immunotoxins constructed with antibody ligands binding to the same receptor and administered simultaneously may act synergistically. Combining immunotoxins targeted toward different tumor antigens may also result in an improved clinical response.

One of the several problems that may be associated with immunotoxin therapy is that cancer cells can become unresponsive due to antigenic loss or mutations in the internalization pathway. 107 Receptor shedding by tumor cells may result in decreased localization and binding to tumor cells and the generation of immune complexes that are potentially nephrotoxic. 106 An anti-interleukin 2 receptor-P. aeruginosa exotoxin A conjugate used to treat human T-cell leukemia caused significant liver toxicity, and neutralizing antibodies were seen within 1 week of systemic administration. 58 Other problems include cross-reactivity of the antibodies with normal cells and the heterogeneity of tumor cells, with regard to antigen expression, which can result in escape of genetic and phenotypic cell variants. 136

As demonstrated in clinical trials against malignant melanoma and breast carcinoma, the immune response mounted against the mouse monoclonal antibody and the toxin may present a difficult problem. 3,7,123 A humoral immune response has been demonstrated to both the antibody and to the toxin in all cases except chronic lymphocytic leukemia. 15,25,47,59,60,127,142 Antibodies to immunotoxins accelerate their plasma clearance and interfere with their cell binding ability and in vitro cytotoxicity. 13,60,61 Antibody ligands from different animal species may stimulate less of a host response than the murine proteins used to produce immunotoxins, and chimeric rodent-human monoclonal antibodies are now being designed that cause a diminished immune reaction and are more effective than their rodent counterparts. 31,118 The use of human monoclonal antibodies, fragments of IgG involved in antigen binding (Fab's), or fragments of IgG digestion with the enzyme pepsin (F(ab')2's) may result in a reduced immune reaction. 123,126 Furthermore, the administration of immunosuppressive drugs such as cyclosporin A or cyclophosphamide may help delay the immune response to immunotoxins and allow repetitive treatment schedules. 5,52

The potential for the CNS to act as an "immunologically privileged site" may offer protection from the host immune system. 145 The half-lives of native ricin A chain immunotoxins are as short as approximately 30 minutes, whereas deglycosylated or unglycosylated ricin A chain immunotoxins have half-lives of 4 to 6 hours. 13,142 Mannosyl receptors on reticuloendothelial system cells and fructose receptors on hepatocytes may be responsible for the rapid clearance of these immunoconjugates from the blood. 13 Production of immunotoxins without oligosaccharides for future clinical trials is possible. The longer half-lives of deglycosylated forms may increase both their capillary penetration and their therapeutic efficacy.

A major potential difficulty of immunotoxin therapy stems from the large size of the molecules and the limited transvascular diffusion into tumor tissue through an intact blood-brain barrier. 51 Blood-brain barrier disruption with the osmotic agent mannitol has been used to treat primary CNS lymphoma with chemotherapeutic drugs and metastatic melanoma to the CNS with monoclonal antibodies. 93,95,96 As previously reported with BCNU, osmotic modification of the blood-brain barrier may be necessary with immunotoxins because of their size, particularly if the treatment of glioblastoma multiforme by the intra-arterial route is contemplated. 48,62 Although Gould, et al., 87 were unable to detect immunotoxin components bound to two breast carcinoma tumor biopsies obtained after treatment, the development of neurotoxicity and the presence of breast cancer epithelial antigen on nerve sheath tissue suggests that the conjugates were able to penetrate into the extravascular space. Conjugates have been made with IgG, Fab's, F(ab')2's, and recombinant forms; reducing the size of the antibody should increase the potential for the CNS to act as an "immunologically privileged site" may offer protection from the host immune system. 145

Although there are few examples of the in vivo treatment of tumors involving the CNS, early results against leptomeningeal neoplasia are encouraging. Clinical trials directed against systemic cancer have demonstrated toxic side effects, such as peripheral neuropathy and capillary-leak syndrome, that may be avoided by an intact blood-brain barrier and the compartmentalized nature of the CNS. Since recombinant human alpha-interferon has been shown to potentiate the action of immunotoxins both in vitro and in vivo against a human ovarian carcinoma cell line, co-instillation of alpha-interferon and immunotoxins into the intrathecal space of patients with carcinomatous meningitis may prove clinically efficacious. 109 In addition, alpha-interferon could be administered with immunotoxins by an intratumoral approach into glioblastomas multiforme, as has been reported previously with interleukin-2 and
lymphokine-activated killer cells. Other agents known to potentiate the effects of immunotoxins in vitro, such as monensin, chloroquine, and the calcium channel-blocking agents verapamil and diltiazem, may be used in combination with immunotoxins if intrathecal or intratumoral delivery is considered.

Conclusions

In this review, we have described the development of immunotoxin therapy as it pertains to the treatment of CNS neoplasia. At the present time, most work has focused on the construction of individual conjugates that are effective in vitro against malignant CNS disease. Future in vitro efforts should include the determination of the most optimal combinations of ligand and toxin and efforts to increase the efficiency of toxin translocation into neoplastic cells. Considerations for improving the clinical effect of immunotoxins might include rendering the patient tolerant or unresponsive to the antibody-toxin conjugate by reducing the immune response via drug administration, by performing total nodal irradiation, or by inducing tolerance to murine and toxin proteins. The establishment of animal models of human neoplastic meningitis with various tumor types known to spread to the CNS and via cerebrospinal fluid pathways is necessary to further document the in vivo efficacy of immunotoxins. Although few advances affecting clinical outcome have been made recently in the field of neuro-oncology, immunotoxins represent a new rapidly evolving treatment modality with considerable potential for basic science research and future clinical application. Investigative avenues are abundant, and only with a persistent focused effort will the applicability of this new therapy for the treatment of CNS neoplasia be determined.

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